Journal Name

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

"InosAminoAcids": Novel Inositol–Amino Acid Hybrid Structures Accessed by Microbial Arene Oxidation

Sarah Pilgrim,[a] Gabriele Kociok-Köhn,[a] Matthew D. Lloyd[b] and Simon E. Lewis[a]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Microbial 1,2-dihydroxylation of sodium benzoate permits rapid construction of novel inositol-amino acid hybrid structures. Both β- and γ-amino acids are accessible by means of an acylnitroso Diels–Alder cycloaddition.

Azacarbasugars are a privileged class of structures for drug development as the amino functionality can modulate biological activity with respect to the parent carbohydrate and replacement of the endocyclic oxygen with carbon confers hydrolytic stability.[1] Azacarbasugar motifs are present in many compounds of medicinal interest. Acarbose **1** and voglibose **2** are α-glucosidase inhibitors used clinically to treat type II diabetes.[2,3] Antibiotic[4] and antifungal[4b,5] properties of azacarbasugars have been reported. The use of *N*-octylvalienamine **3** and its 4-epimer as therapies for Gaucher disease and G_{M1}-gangliosidosis is under investigation.[6] In addition, the anti-influenza drug oseltamivir **4** may also be considered to be an azacarbasugar (Figure 1).[7]

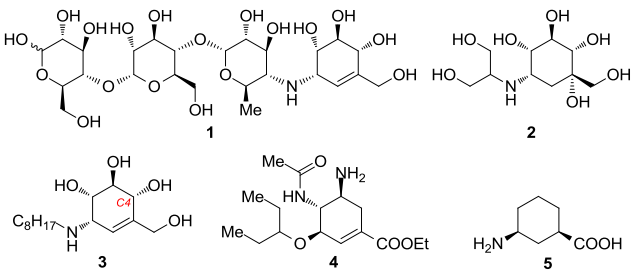
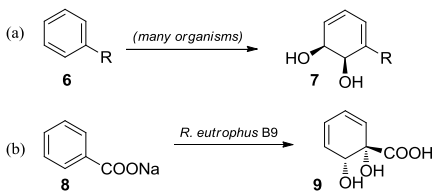


Fig. 1 Representative azacarbasugars and cyclic amino acids.

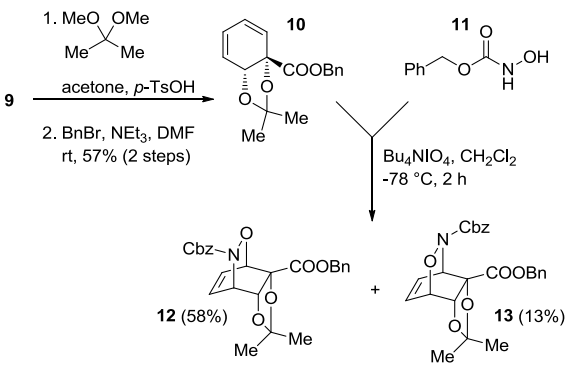
In the field of peptide engineering, incorporation of non-natural β- and γ- amino acids has been employed to furnish peptides with designed properties.[8] Constrained cyclic amino acids are effective for imparting secondary structure to peptides and the cyclohexane γ-amino acid **5** has been employed for the construction of peptide nanotubes with hydrophobic cavities.[9] Polyhydroxylated analogues of **5** would permit control of the hydrophobicity of such cavities and allow for modified secondary structures based on additional hydrogen bonding interactions.

Enzymatic dihydroxylation of arenes to produce enantiopure building blocks for synthesis is well established methodology.[10] For dihydroxylation of monosubstituted arenes, the most common regiochemical outcome is installation of the diol *ortho,meta* to the pre-existing substituent (**7**, Scheme 1a). However, *R. eutrophus* B9[11,†] and certain other organisms[12] are able to metabolise benzoates such that the diol is introduced *ipso,ortho* to the carboxy functionality (Scheme 1b). The chiron **9** derived from the oxidation of benzoate has found diverse synthetic applications[13] and we have recently demonstrated access to new reaction manifolds by means of tricarbonyliron complexes of **9**.[14] Arene diols are ideal starting materials for azacarbasugar synthesis; *ortho,meta* diols of type **7** have been utilised in this context.[15] In contrast, *ipso,ortho* diols of type **9** have remained unexploited to date. We targeted efficient access to C-substituted azacarbasugar structures from **9**, made possible by the pre-existing quaternary centre. Specifically, we sought to access C-carboxy inosamines ("InosAminoAcids"), a hitherto unknown class of compound.



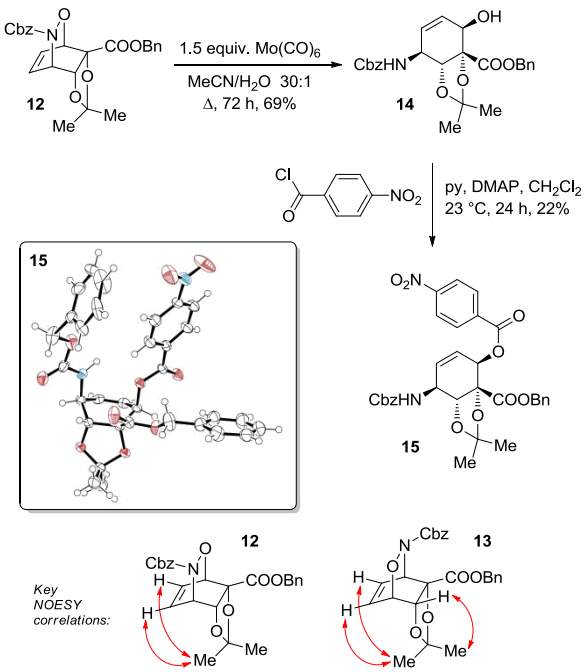
Scheme 1 Regio- and stereoselectivity of dioxygenases.

Formation of the known[13a,g] acetonide of **9**, followed by carboxylate benzylation afforded protected diene **10**, which was employed in an acylnitroso cycloaddition. The dienophile was generated *in situ* by the action of tetrabutylammonium periodate on *N*-(benzyloxycarbonyl)hydroxylamine **11**.[15h,16] Selectivity in cycloadditions employing arene diol-derived dienes has been extensively studied[17] and precedent suggested that approach of the dienophile to the diene face opposite the acetonide would be favoured.[13a,15h,16b,c,18] In the event, only two of four possible regioisomers were isolated (**12** and **13**, Scheme 2).

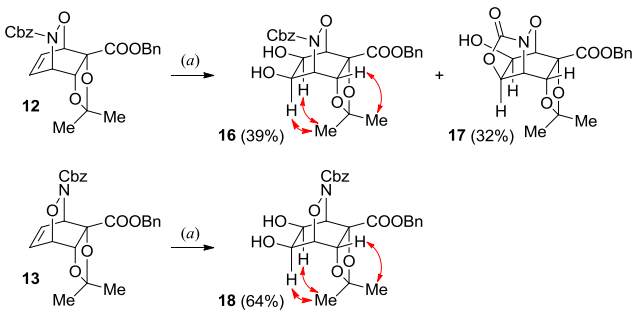


Scheme 2 Acylnitroso cycloaddition.

The major product of the cycloaddition (**12**) was that in which the Cbz group was introduced distal to the benzyl ester, which we attribute to decreased steric hindrance with respect to formation of **13**. Major adduct **12** was treated with molybdenum hexacarbonyl to effect selective N–O bond scission, followed by formation of crystalline *p*-nitrobenzoate derivative **15**. The absolute structure of **15** was confirmed by X-ray crystallography, from which the structure of **12** was inferred. The same sequence of transformations did not furnish a crystalline derivative when applied to minor adduct **13**. Thus, the structure of **13** was elucidated by means of NOESY correlations.‡ Specifically, an interaction between the olefinic protons and the acetonide endo methyl protons was observed for both **12** and **13**; such an interaction would not be expected for cycloadducts arising from dienophile approach to the diene face bearing the acetonide.

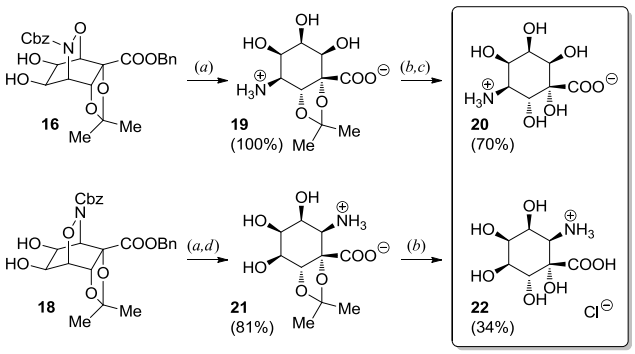


Scheme 3 Structural elucidation of **12** and **13**. NOESY correlations are shown with double-headed arrows. ORTEP Diagram of **15** shows ellipsoids at 50% probability. Solvent and disorder in the Cbz phenyl ring are omitted for clarity. H atoms are shown as spheres of arbitrary radius.



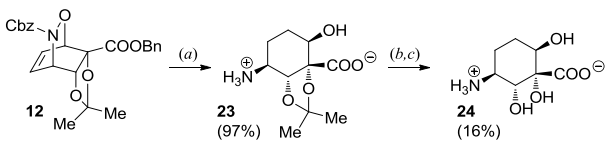
Scheme 4 (*a*) NMO, cat. OsO_4, acetone/H_2O 4:1, 24 h, rt. NOESY correlations are shown with double-headed arrows.

Cycloadducts **12** and **13** were subjected to Upjohn dihydroxylation conditions, affording in each instance the diol corresponding to approach of the oxidant to the less hindered face of the olefin. Stereochemistry of addition was again elucidated by NOESY correlation‡ (Scheme 4). For both diols **16** and **18**, interaction of the acetonide endo methyl protons with the hydroxyl group methines was observed, indicative of the axial orientation of the methines and, by inference, the equatorial orientation of the hydroxyl groups. In the dihydroxylation of **12**, unexpected cyclic carbamate **17** was also formed. Hydrogenolysis of diols **16** and **18** effected multiple reductive operations cleanly, allowing access to the target inosaminoacids **20** and **22** (Scheme 5) simply by acetonide removal in aqueous hydrochloric acid.[19]



Scheme 5 (*a*) H_2, Pd/C, MeOH, 24 h, rt. (*b*) 1 M $HCl_{(aq)}$, 24 h, rt. (*c*) C_{18} reversed-phase chromatography. (*d*) Trituration with EtOH

In adddition to inosaminoacids **20** and **22**, less highly oxygenated structures are also accessible via the acylnitroso cycloaddition reported here. For example, major cycloadduct **12** could be subjected directly to hydrogenolysis/acetonide cleavage as above, in this instance giving rise to dihydro-3-C-carboxy-*ent*-conduramine A1 (**24**, Scheme 6).[20] Polyhydroxylated zwitterionic species such as **19**-**24** are known to be difficult to purify; repeated chromatography was required in some instances.[19]



Scheme 6 (*a*) H_2, Pd/C, MeOH, 24 h, rt. (*b*) 1 M $HCl_{(aq)}$, 24 h, rt. (c) C_{18} reversed-phase chromatography.

Conduramine derivative **24** was highly crystalline and submitted to X-ray crystallography (Figure 2), providing further confirmation for the assignment of **12** and **13**. It is noteworthy that in the solid state **24** adopts a near-perfect chair conformation.

Fig. 2 ORTEP Diagram of **24** shows ellipsoids at 50% probability. H atoms are shown as spheres of arbitrary radius.

Inosaminoacids **20** and **22** and conduramine derivative **24** were evaluated for inhibition of glycosidase activity[21] against α-glucosidase (type I from Baker's yeast), β-D-glucosidase (almond), β-galactosidases (from *A. oryzae* and *E. coli*) and β-D-glucuronidases (from bovine liver, *E. coli* and *P. vulgata*); no inhibitory activity was observed.

In summary, we have described a very concise synthetic route

to a novel class of azacarbasugar. Inosaminoacids **20** and **22** contain six contiguous stereocentres (including a quaternary centre), yet are accessed in just seven steps from sodium benzoate. Current work in our laboratory concerns accessing inosaminoacids having alternative stereochemistries and their incorporation into oligopeptides.

We gratefully acknowledge EPSRC (vacation bursary to S.P.) and the University of Bath for funding. We thank Prof. Andrew G. Myers (Harvard) for a generous gift of *R. eutrophus* B9 cells. We also thank the EPSRC national mass spectrometry service centre for analyses. We are grateful to Prof. J. Grant Buchanan (Bath) for helpful discussions.

Notes and references

[a] *Department of Chemistry, University of Bath, Bath, BA2 7AY, UK. Fax:+44 (0)1225 386231; Tel:+44 (0)1225 386568; E-mail: S.E.Lewis@bath.ac.uk*

[b] *Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, UK.*

† Formerly known as *Alcaligenes eutrophus* B9.

‡ Electronic Supplementary Information (ESI) available: Experimental procedures, characterisation data and ^{1}H NMR and ^{13}C NMR spectra for all novel compounds, as well as selected 2D-NMR data. Crystallographic data for **15** and **24** (CCDC 809598 and 809599). For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b000000x/

1 For reviews, see: (a) O. Arjona, A. M. Gómez, J. C. López and J. Plumet, *Chem. Rev.*, 2007, **107**, 1919.

2 (*a*) C. Rosak and G. Mertes, *Curr. Diabetes Rev.*, 2009, **5**, 157; (*b*) A. Godbout and J.-L. Chiasson, *Curr. Diabetes Rep.*, 2007, **7**, 333; (*c*) U. F. Wehmeier and W. Piepersberg, *Appl. Microbiol. Biotech.*, 2004, **63**, 613.

3 (*a*) S. Ogawa, M. Kanto and Y. Suzuki, *Mini-Rev. Med. Chem.*, 2007, **7**, 679; (*b*) X. Chen, Y. Zheng and Y. Shen, *Curr. Med. Chem.*, 2006, **13**, 109; (*c*) H.-P. Zhou and X.-Y. Chen, *Zhongguo Yiyao Gongye Zazhi*, 2006, **37**, 574; (*d*) S. Horii, *Takeda Kenkyushoho*, 1993, **52**, 1.

4 (*a*) T. Mahmud, *Nat. Prod. Rep.*, 2003, **20**, 137; (*b*) T. Iwasa, *Takeda Kenkyushoho*, 1978, **37**, 307; (*c*) T. Iwasa, H. Yamamoto and M. Shibata, *J. Antibiot.*, 1970, **23**, 595; (*d*) M. P. Mingeot-Leclercq, Y. Glupczynski and P. M. Tulkens, *Antimicrob. Agents Chemother.*, 1999, **43**, 727; (*e*) E. J. Begg and M. L. Barclay, *Brit. J. Clin. Pharmaco.*, 1995, **39**, 597.

5 (*a*) A. P. J. Trinci, *Br. Mycol. Sy.*, 1983, **9**, 113; (*b*) L. Vértsey, H.-W. Fehlhaber and A. Schulz, *Angew. Chem. Int. Edn. Engl.*, 1994, **33**, 1844.

6 Y. Suzuki, S. Ogawa and Y. Sakakibara, *Persp. Med. Chem.*, 2009, **3**, 7.

7 (*a*) J. Magano, *Chem. Rev.*, 2009, **109**, 4398; (*b*) L. Werner, A. Machara and T. Hudlický, *Adv. Synth. Catal.*, 2010, **352**, 195; (*c*) B. Sullivan, I. Carrera, M. Drouin and T. Hudlický, *Angew. Chem. Int. Edn. Engl.*, 2009, **48**, 4229; (*d*) M. Matveenko, A. C. Willis and M. G. Banwell, *Tetrahedron Lett.*, 2008, **49**, 7018; *ibid.* 2009, **50**, 2982; (*e*) J.-J. Shie, J.-M. Fang and C.-H. Wong, *Angew. Chem. Int. Edn. Engl.*, 2008, **47**, 5788.

8 D. Seebach and J. Gardiner, *Acc. Chem. Res.*, 2008, **41**, 1366.

9 M. Amorín, L. Castedo and J. R. Granja, *J. Am. Chem. Soc.* 2003, **125**, 2844.

10 For reviews, see: (*a*) T. Hudlický and J. W. Reed, *Synlett*, 2009, 685; (*b*) D. R. Boyd and T. D. H. Bugg, *Org. Biomol. Chem.*, 2006, **4**, 181; (*c*) R. A. Johnson, *Org. React.*, 2004, **63**, 117; (*d*) T. Hudlický, D. Gonzales and D. T. Gibson, *Aldrichimica Acta*, 1999, **32**, 35.

11 (*a*) A. M. Reiner and G. D. Hegeman, *Biochem.*, 1971, **10**, 2530. (*b*) H.-J. Knackmuss and W. Reineke, *Chemosphere*, 1973, **2**, 225; (*c*) W. Reineke and H.-J. Knackmuss, *Biochim. Biophys. Acta*, 1978, **542**, 412; (*d*) W. Reineke, W. Otting and H.-J. Knackmuss, *Tetrahedron*, 1978, **34**, 1707; (*e*) K.-H. Engesser, E. Schmidt and H.-J. Knackmuss, *Appl. Environ. Microbiol.*, 1980, **39**, 68.

12 (*a*) J. T. Rossiter, S. R. Williams, A. E. G. Cass and D. W. Ribbons, *Tetrahedron Lett.*, 1987, **28**, 5173; (*b*) M. G. Banwell, A. J. Edwards, D. W. Lupton and G. Whited, *Aust. J. Chem.*, 2005, **58**, 14; (*c*) S.-Y. Sun, X. Zhang, Q. Zhou, J.-C. Chen and G.-Q. Chen, *Appl. Microbiol. Biotechnol.*, 2008, **80**, 977.

13 (*a*) G. N. Jenkins, D. W. Ribbons, D. A. Widdowson, A. M. Z. Slawin and D. J. Williams, *J. Chem. Soc., Perkin Trans 1*, 1995, 2647; (*b*) A. G. Myers, D. R. Siegel, D. J. Buzard and M. G. Charest, *Org. Lett.*, 2001, **3**, 2923; (*c*) M. H. Parker, B. E. Maryanoff and A. B. Reitz, *Synlett*, 2004, 2095; (*d*) M. D. Mihovilovic, H. G. Leisch and K. Mereiter, *Tetrahedron Lett.*, 2004, **45**, 7087; (*e*) M. G. Charest, C. D. Lerner, J. D. Brubaker, D. R. Siegel and A. G. Myers, *Science*, 2005, **308**, 395; (*f*) M. G. Charest, D. R. Siegel and A. G. Myers, *J. Am. Chem. Soc.* 2005, **127**, 8292; (*g*) T. C. M. Fischer, H. G. Leisch and M. D. Mihovilovic, *Monatsh. Chem.*, 2010, **141**, 699.

14 (*a*) M. Ali Khan, M. F. Mahon, A. J. W. Stewart and S. E. Lewis, *Organometallics*, 2010, **29**, 199; (*b*) M. Ali Khan, J. P. Lowe, A. L. Johnson, A. J. W. Stewart and S. E. Lewis, *Chem. Commun.*, 2011, **47**, 215.

15 (*a*) L. Werner, J. Reed Hudlický, M. Wernerova and T. Hudlický, *Tetrahedron*, 2010, **66**, 3761; (*b*) F. Fabris, J. Collins, B. Sullivan, H. Leisch and T. Hudlický, *Org. Biomol. Chem.*, 2009, **7**, 2619; (*c*) B. J. Paul, J. Willis, T. A. Martinot, I. Ghiviriga, K. A. Abboud and T. Hudlický, *J. Am. Chem. Soc.*, 2002, **124**, 10416; (*d*) B. J. Paul, T. A. Martinot, J. Willis and T. Hudlický, *Synthesis*, 2001, 952; (*e*) K. A. Oppong, T. Hudlický, F. Yan, C. York and B. V. Nguyen, *Tetrahedron*, 1999, **55**, 2875; (*f*) M.-C. Lallemand, M. Desjardins, S. Freeman and T. Hudlický, *Tetrahedron Lett.*, 1997, **38**, 7693; (*g*) T. Hudlický, K. A. Abboud, J. Bolonick, R. Maurya, M. L. Stanton and A. J. Thorpe, *Chem. Commun.*, 1996, 1717; (*h*) T. Hudlický, H. F. Olivo and B. McKibben, *J. Am. Chem. Soc.*, 1994, **116**, 5108; (*i*) H. A. J. Carless and S. S. Malik, *Tetrahedron: Asymmetry*, 1992, **3**, 1135.

16 (*a*) S. Iwasa, A. Fakhruddin and H. Nishiyama, *Mini-Rev. Org. Chem.*, 2005, **2**, 157; (*b*) T. Hudlický and H. F. Olivo, *J. Am. Chem. Soc.*, 1992, **114**, 9694; (*c*) T. Hudlický and H. F. Olivo, *Tetrahedron Lett.*, 1991, **32**, 6077; (*d*) G. W. Kirby, H. McGuigan, J. W. M. Mackinnon, D. McLean and R. P. Sharma, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1437.

17 (*a*) S. M. Ogbomo and D. J. Burnell, *Org. Biomol. Chem.*, 2006, **4**, 3838; (*b*) J. R. Gillard and D. J. Burnell, *Can. J. Chem.*, 1992, **70**, 1296; (*c*) J. R. Gillard and D. J. Burnell, *J. Chem. Soc., Chem. Commun.*, 1989, 1439.

18 (*a*) C. K. Jana, S. Grimme and A. Studer, *Chem. Eur. J.*, 2009, **15**, 9078; (*b*) K.-W. Lin, Y.-C. Wang and T.-H. Yan, *J. Chin. Chem. Soc.-Taip.*, 2008, **55**, 418; (*c*) D. Gamenara, G. Seoane, H. Heinzen and P. Moyna, *Lat. Am. J. Pharm.*, 2008, **27**, 34; (*d*) Y. Yamamoto and H. Yamamoto, *Patent* WO2005/068457; (*e*) S. Elango and T.-H. Yan, *J. Org. Chem.*, 2002, **67**, 6954; (*f*) S. Elango, Y.-C. Wang, C.-L. Cheng and T.-H. Yan, *Tetrahedron Lett.*, 2002, **43**, 3757; (g) H. Noguchi, T. Aoyama and T. Shioiri, *Heterocycles*, 2002, **58**, 471; (*h*) H. Noguchi, T. Aoyama and T. Shioiri, *Tetrahedron Lett.*, 1997, **38**, 2883; (*i*) K. Schürrle, B. Beier and W. Piepersberg, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2407; (*j*) M. F. Mahon, K. Molloy, C. A. Pittol, R. J. Pryce, S. M. Roberts, G. Ryback, V. Sik, J. O. Williams and J. A. Winders, *J. Chem. Soc., Perkin Trans. 1*, 1991, 1255; (*k*) P. J. Geary, R. J. Pryce, S. M. Roberts, G. Ryback and J. A. Winders, *J. Chem. Soc., Chem. Commun.*, 1990, 204; (*l*) C. A. Pittol, R. J. Pryce, S. M. Roberts, G. Ryback, V Sik. and J. O. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1989, 1160; (*m*) I. C. Cotterill, S. M. Roberts and J. O. Williams, *J. Chem. Soc., Chem. Commun.*, 1988, 1628.

19 γ-Inosaminoacid **20** and conduramine derivative **24** required purification by C_{18} reversed-phase chromatography after acetonide removal. In contrast, β-inosaminoacid **22** could be isolated as the pure hydrochloride without chromatography, simply by removal of solvent; precursor **21** had been purified by trituration with EtOH, a procedure specific to this compound.

20 R. Łysek and P. Vogel, *Tetrahedron*, 2006, **62**, 2733.

21 A. L. Ball, K. A. Chambers, M. Hewinson, S. Navaratanarajah, L. Samrin, N. Thomas, A. H. Tyler, A. J. Wall and M. D. Lloyd, *J. Enzym. Inhib. Med. Chem.*, 2008, **23**, 131.